(FILE 'HOME' ENTERED AT 11:40:23 ON 16 APR 2003)

FILE 'MEDLINE, CAPLUS, BIOSIS, AGRICOLA' ENTERED AT 11:40:27 ON 16 APR 2003

L1 202587 S METHANOL
L2 48 S L1 AND CULTURE AND INACTIVE
L3 25 DUP REM L2 (23 DUPLICATES REMOVED)

FILE 'STNGUIDE' ENTERED AT 11:43:45 ON 16 APR 2003

FILE 'MEDLINE, CAPLUS, BIOSIS, AGRICOLA' ENTERED AT 12:39:17 ON 16 APR 2003

L4 3515 S GALACTOSE (2N) OXIDASE
L5 78 S L4 AND (OXIDIZING)
L6 8 S L5 AND CATALYTIC
L7 5 DUP REM L6 (3 DUPLICATES REMOVED)

FILE 'STNGUIDE' ENTERED AT 12:40:22 ON 16 APR 2003

(FILE 'HOME' ENTERED AT 11:40:23 ON 16 APR 2003)

FILE 'MEDLINE, CAPLUS, BIOSIS, AGRICOLA' ENTERED AT 11:40:27 ON 16 APR 2003

L1 202587 S METHANOL
L2 48 S L1 AND CULTURE AND INACTIVE
L3 25 DUP REM L2 (23 DUPLICATES REMOVED)

FILE 'STNGUIDE' ENTERED AT 11:43:45 ON 16 APR 2003

FILE 'MEDLINE, CAPLUS, BIOSIS, AGRICOLA' ENTERED AT 12:39:17 ON 16 APR 2003

L4 3515 S GALACTOSE (2N) OXIDASE
L5 78 S L4 AND (OXIDIZING)
L6 8 S L5 AND CATALYTIC
L7 5 DUP REM L6 (3 DUPLICATES REMOVED)

FILE 'STNGUIDE' ENTERED AT 12:40:22 ON 16 APR 2003

FILE 'MEDLINE, CAPLUS, BIOSIS, AGRICOLA' ENTERED AT 13:01:03 ON 16 APR 2003

L8 340 S METHANOL (3N) INDUCTION
L9 8 S L8 (10N) TEMPERATURE
L10 4 DUP REM L9 (4 DUPLICATES REMOVED)

FILE 'STNGUIDE' ENTERED AT 13:02:05 ON 16 APR 2003

FILE 'CAPLUS' ENTERED AT 13:03:23 ON 16 APR 2003

FILE 'MEDLINE, CAPLUS, BIOSIS, AGRICOLA' ENTERED AT 13:03:26 ON 16 APR 2003

L11 246 S METHANOL AND INDUCTION AND TEMPERATURE
L12 17 S L11 AND PICHIA
L13 10 DUP REM L12 (7 DUPLICATES REMOVED)
L14 118 S L11 AND (25 OR DECREAS? OR LOW?)
L15 97 DUP REM L14 (21 DUPLICATES REMOVED)
L16 4 S L15 AND PICHIA
L17 1478 S METHANOL AND INDUC? AND TEMPERATURE
L18 31 S L17 AND PICHIA
L19 14 S L18 AND (25 OR DECREA? OR LOW?)
L20 8 DUP REM L19 (6 DUPLICATES REMOVED)

ANSWER 4 OF 5 CAPLUS COPYRIGHT 2003 ACS

AN 1993:18335 CAPLUS

DN 118:18335

TI Preparation of fully oxidized active and reduced inactive forms of **galactose oxidase** from Dactylium dendroides using ferricyanide-containing **oxidizing** and ferrocyanide-containing reducing forms of ion exchange resins

AU Montague-Smith, Michael P.; Wachter, Rebekka M.; Branchaud, Bruce P.

CS Dep. Chem., Univ. Oregon, Eugene, OR, 97403, USA

SO Analytical Biochemistry (1992), 207(2), 353-5
CODEN: ANBCA2; ISSN: 0003-2697

DT Journal

LA English

AB **Galactose oxidase** (EC 1.1.3.9) is a type II mononuclear copper protein secreted by the fungus Dactylium dendroides. The enzyme catalyzes the oxidn. of primary alcs. with O2, producing aldehydes and H2O2. The details of the **catalytic** mechanism have not been fully elucidated. A chronic problem in kinetic assays of **galactose oxidase** is the tendency of the enzyme to exist as a mixt. of oxidized active and one-electron reduced inactive forms. The two forms cannot be phys. sepd. by std. purifn. techniques. The enzyme can be activated by treatment with one-electron oxidants. This treatment results in maximally active enzyme. Once the active enzyme has been formed, the **oxidizing** agent must be removed, since it can substitute for the natural oxidant, dioxygen, in the enzyme reaction and alter reaction rates. Dialysis is tedious and does not lead to consistently reproducible results. Desalting columns are more effective but are not sufficiently consistent. Finally, activated enzyme is slowly reduced to a mixt. of active and inactive forms, so that a large quantity of identical enzyme necessary for extensive kinetic analyses cannot be maintained. A method of rapidly activating or deactivating small samples of enzyme to a consistent specific activity was developed. Ferricyanide and ferrocyanide, highly charged redox active anions, bind strongly to anion exchange resins, producing redox-active resins that are capable of **oxidizing** or reducing **galactose oxidase** to provide the active or inactive forms of the enzyme. The redox resins are easily made and stable and give reproducibly active or inactive enzyme.

L8 ANSWER 14 OF 18 CAPLUS COPYRIGHT 2003 ACS
AN 1997:685007 CAPLUS
DN 127:358067
TI Online monitoring and control of **methanol** concentration in shake-flask **cultures** of Pichia pastoris
AU Guarna, M. M.; Lesnicki, G. J.; Tam, B. M.; Robinson, J.; Radziminski, C. Z.; Hasenwinkle, D.; Boraston, A.; Jervis, E.; MacGillivray, R. T. A.; Turner, R. F. B.; Kilburn, D. G.
CS Biotechnology Laboratory, University of British Columbia, Vancouver, BC, V6T 1Z3, Can.
SO Biotechnology and Bioengineering (1997), 56(3), 279-286
CODEN: BIBIAU; ISSN: 0006-3592
PB Wiley
DT Journal
LA English
AB The methylotrophic **yeast** P. pastoris can be used to express recombinant genes at high levels under the control of the MeOH-**inducible** alc. **oxidase** 1 (AOX1) **promoter**. Accurate regulation of the MeOH concn. in P. pastoris **cultures** is necessary to maintain induction, while preventing accumulation of MeOH to cytotoxic levels. An inexpensive MeOH sensor that uses a gas-permeable silicone rubber tube immersed in the **culture** medium and an org. solvent vapor detector was developed. The sensor was used to monitor MeOH concn. continuously throughout a fed-batch shake-flask **culture** of a P. pastoris clone producing the N-lobe of human transferrin. The sensor calibration was stable for the duration of the **culture** and the output signal accurately reflected the MeOH concn. detd. off-line by HPLC. A closed-loop control system utilizing this sensor was developed and used to maintain a 0.3% MeOH concn. in the **culture**. Use of this system resulted in a 5-fold increase in volumetric protein productivity over levels obtained using the conventional fed-batch protocol.

L15 ANSWER 1 OF 1 MEDLINE DUPLICATE 1
AN 96292476 MEDLINE
DN 96292476 PubMed ID: 8728322
TI Expression and secretion of rabbit plasma cholesteryl ester transfer protein by Pichia pastoris.
AU Kotake H; Li Q; Ohnishi T; Ko K W; Agellon L B; Yokoyama S
CS Lipid and Lipoprotein Research Group, University of Alberta, Edmonton, Canada.
SO JOURNAL OF LIPID RESEARCH, (1996 Mar) 37 (3) 599-605.
Journal code: 0376606. ISSN: 0022-2275.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199611
ED Entered STN: 19961219
Last Updated on STN: 19990129
Entered Medline: 19961125
AB The rabbit cholesteryl ester transfer protein (CETP) was expressed in the methylotrophic yeast Pichia pastoris by introducing the CETP cDNA under the control of the **methanol-inducible** alcohol oxidase **promoter**. The cDNA was cloned from in vitro amplified cDNA of rabbit liver mRNA. The nucleotide sequence of the cloned cDNA differed slightly from the previously published sequence that changed the amino acid sequence in six residues. Interestingly, five of these replacements are identical to the corresponding residues in human CEPT. In addition, the encoded mature N-terminal sequence was changed from Cys- to Arg-Glu-Phe- to link the CETP sequence to the yeast acid phosphatase signal peptide. The culture medium of the transformed cells induced with 1% methanol contained both cholesteryl ester and triglyceride transfer activity comparable to that of rabbit plasma. Like rabbit plasma, the lipid transfer activity in the medium could be inhibited by monoclonal antibodies that block CE/TG transfer or TG transfer alone. Immunoblot analysis of M(r) = 80 K and minor species of M(r) = 60-100 K. In spite of these differences, the specific transfer activity of the recombinant CETP was indistinguishable from that of rabbit plasma CETP of M(r) = 74 K. N-Glycosidase F treatment converted both the recombinant and plasma CETP to a single species of M(r) = 55 K. Both the plasma and recombinant CETP lost their activity after removal of N-linked carbohydrate and sialic acid. A single 55 K component was found in the cell-lysates. The intracellular form of the recombinant CETP was not modified by N-glycosidase F treatment. In conclusion, the recombinant CETP is synthesized as an **inactive** polypeptide that is processed and secreted as a functional glycoprotein. In addition, the N-terminal Cys residue of the plasma CETP is not required for its activity.